RESOLUTION OF DL-PROLIN AND DL-HYDROXY-PROLIN

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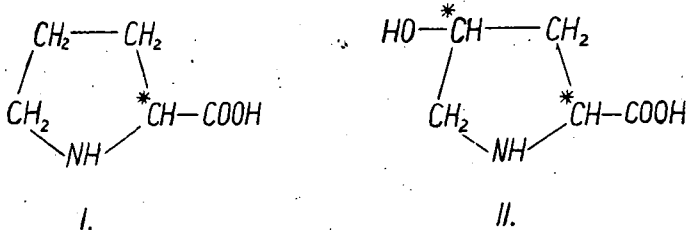
Successful attempts to resolve DL-prolin and DL-hydroxy-prolin, respectively, as their 3,5-dinitro-benzoates with the use of brucin, in satisfactory yields and in an adequate optical purity, are described.

In the course of our research work it arose the necessity to evolve a reliable, reproducible and identically developed method for the chemical resolution of both DL-prolin (I) and DL-hydroxyprolin (II).



I. II.

Fig. 1

FISCHER and ZEMPLÉN [1] were the first in describing the chemical resolution of DL-prolin (I), on separating m-nitrobenzoyl-DL-prolin by cinchonine. Another description originates from VELLUZ, AMIARD and HEYNÉS [2], who resolved the 3,5-dinitrobenzoate of the compound in question with D(—) threo-1-(p-nitrophenyl)-2-amino-propane-1,3-diol. The separation of DL-hydroxy-prolin (II) based on similar principles was published simultaneously as well [2]. This latter compound was, in form of its phenyl-ureo derivative, resolved with quinine earlier by LEUCHS and BREWER [3], and, respectively, by LEUCHS and BORMANN [4].

While the use of earlier methods [1], [3], [4] is impeded by certain properties of the intermediates (poor crystallization and, respectively, difficulties of decomposition), the applicability of the recent process [2] is limited by the expensiveness of the resolving agent.

Considering the mentioned points of view, the 3,5-dinitrobenzoyl derivatives [2] easy to prepare and ready to crystallize have been chosen as intermediates whereas brucin, a cheap substance has been used as a resolv-

ing agent. The corresponding D-3,5-dinitrobenzoyl brucin salt as well as L-3,5-dinitrobenzoyl brucin salt of L-hydroxy-prolin of both compounds studied was succesfully isolated while the L-modification of L-prolin contained by the mother liquor was re-converted into the L-3,5-dinitrobenzoyl derivative without crystallization. In the case of the L-prolin derivative 3,5-dinitrobenzoate was refined until an adequate rotation value was attained [2]. By this way, we succeeded in obtaining optically pure D- and L-prolin with values $[\alpha]_D^{20} = +81°$ and $-82°$, respectively, in overall yields of 46,5 and 43 %, respectively (referred to the half amount of initial DL-substances), further D- and L-hydroxy-prolin with values $[\alpha]_D^{20} = +77{,}0°$ and $-76{,}0°$, respectively, in overall yields of 45,2 and 44,8 %, respectively.

Experimental

DL-prolin (I), prepared from diethyl malonate and acryl nitrile according to ALBERTSON and FILLMAN [5], m. p. 210—211°C.

3,5-Dinitrobenzoyl-DL-prolin, prepared from 3,5-dinitrobenzoyl chloride and DL-prolin, according to reference [2], in 92 % yield, m. p. 221—222°C [2].

3,5-Dinitrobenzoyl-D-prolin. The filtered solution of 15,84 g of 3,5-dinitrobenzoyl-DL-prolin in 100 ml of acetone was treated with the filtered solution of 24,0 g of brucin.$4H_2O$ in 100 ml of acetone. On removing the solvent by distillation, the residual reddish yellow crystalline substance was dissolved in 450 ml of hot water, filtered and allowed to stand at room temperature, then at +3°C overnight. Recrystallization from water afforded (from 18,37 g of yellow platelets) 17,54 g of 3,5-dinitrobenzoyl-D-prolin brucinate, m. p. 111—112°C. $[\alpha]_D^{22} = +31{,}5°$ (c: 1,018; waterfree ethanol). Analysis of substance dried for 3 hours at 80°C under a pressure of 30 mm Hg. Calculated from formula $C_{35}H_{37}O_{11}N_5$, N 9,95; found 9,94 %.

The solution of 17,54 g of 3,6-dinitrobenzoyl-D-prolin brucinate in 486 ml of hot water was made slightly alkaline with a 1,0 N solution of sodium hydroxyde, the precipitated brucin base (8,4 g) filtered on standing for a few hours at +3°C and the residual brucin base removed by extracting the aqueous solution with 3 × 200 ml portions of ether. On removing ether, the aqueous solution was adjusted with concentrated hydrochloric acid to pH 6, allowed to stand for 24 hours at +3°C, the yellow crystals (4,1 g; m. p. 174—176°C) filtered and the mother liquor concentrated to 75 ml (yield: further 1,95 g of substance). Recrystallization from water afforded 4,9 g (62 %) of 3,5-dinitrobenzoyl-D-prolin of adequate purity, m. p. 179°C [2]. $[\alpha]_D^{30} = +94{,}8°$ (c: 1,292; 50 % ethanol).

D-prolin (I), prepared by hydrolysis with 5 N hydrochloric acid and liberated with Amberlite IR—4B ion exchange resin, by the VELLUZ method [2], in 82 % yield, m. p. 215—220°C (decomp.). $[\alpha]_D^{20} = +81°$ (c: 0,500, H_2O) [1], [2].

3,5-Dinitrobenzoyl-L-prolin. On concentrating the mother liquor obtained in the previous operations, containing mainly 3,5-dinitrobenzoyl-L-prolin to a volume of 200 ml, the precipitated crystalline substance (0,42 g; m. p. 135—

150°C) was filtered, the filtrate made alkaline with 1,0 N sodium hydroxyde and allowed to stand for a few hours at +3°C. The precipitated brucin base (11.06 g) was filtered, the aqueous phase extracted with 3 × 100 ml portions of ether, adjusted with concentrated hydrochloric acid to pH 6, allowed to stand a few hours at +3°C, the crystalline substance filtered (4,26 g; m. p. 172—174°C) and the mother liquor concentrated to one third of original volume, thus affording further 2,37 g of substance. On repeated recrystallisation from water, yield 4,46 g (59 %) of 3,5-dinitrobenzoyl-L-prolin of satisfactory purity, m. p. 178—179°C [2]. $[\alpha]_D^{24} = -94{\cdot}7°$ (*c*: 1,015; 50 % ethanol).

L-prolin (I) prepared as the D-modification. Yield 79 %, m. p. 220—222°C (decomp.). $[\alpha]_D^{20} = -82°$ (*c*: 0,500, H_2O) [1], [2].

DL-hydroxy-prolin (II), prepared by the GAUDRY and GODIN method [6] from allyl chloride and diethyl malonate, m. p. 255°C.

3,5-Dinitrobenzoyl-DL-hydroxy-prolin. 3,5-Dinitrobenzoyl chloride (9,0 g) was added in small portions, under continous stirring at 0°C to the solution of 5 g of DL-hydroxy-prolin in 50 ml of 1,0 N sodium hydroxyde. An alkaline medium was maintained during the reaction by adding dropwise further quantities of 1,0 N sodium hydroxide (total further addition: 30 ml of 1,0 N sodium hydroxyde). On termination of the reaction (about 150 minutes), the yellow liquid was acidified with concentrated hydrochloric acid (17 ml), the aqueous phase allowed to stand until solidification of the precipitate (24 hours). On filtering and washing with some cold water and with 2 × 25 ml portions of ether afforded 11,78 g of 3,5-dinitrobenzoyl-DL-hydroxy-prolin as white crystals, m. p. 203—206°C (95 %) [2].

3,5-Dinitrobenzoyl-D-hydroxy-prolin. 3,5-dinitrobenzoyl-DL-hydroxy-prolin (14 g) and 20,02 g of brucin.$4H_2O$ was dissolved in 250 ml of hot water, filtered, allowed to cool, a seed crystal added and allowed to stand for a day at room temperature. The mother liquor decanted carefully from the settled crystals forming yellow platelets, suspended in 40 ml of ethanol and filtered (16,2 g). On recrystallization from 130 ml of hot water afforded 13 g (84 %) of 3,5-dinitrobenzoyl-D-hydroxy-prolin brucinate, m. p. 147—150°C. $[\alpha]_D^{20} = +54°$ (*c*: 0,500; abs. ethanol).

The obtained amount (13 g) of D-brucinate was dissolved in 55 ml of 1,0 N sodium hydroxyde, cooled, the precipitated brucin base filtered and the brucin extracted with 2 × 10 ml of 1,0 N sodium hydroxide. On acidifying the aqueous phase with 12 ml of concentrated hydrochloric acid, extracting the acid solution with 6 × 100 ml portions of ethylacetate, drying the organic phase and removing the solvent by distillation, 3,5-dinitrobenzoyl-D-hydroxy-prolin was obtained in form of a solid foam. Yield: 5,16 g (89 %). $[\alpha]_D^{20} = +147°$ (*c*: 1,004; 50 % ethanol).

D-Hydroxy-prolin (II). 3,5-dinitrobenzoyl-D-hydroxy-prolin was refluxed with 90 ml of 5 N hydrochloric acid, allowed to stand for 4 hours, the separated 3,5-dinitro-benzoic acid collected and the filtrate extracted with 2 × 120 ml portions of ether. Having evaporated the decolorized aqueous phase, under reduced pressure to dryness, the residue was dissolved in 10 ml hot water,

then decolorized again and to the clear hot solution the mixture of 50 ml of abs. ethanol and 3 ml of fresh distilled anilin was added. After standing for a day at 0°C the separated crystals filtered, washed with waterfree ethanol and ether and dried, yield 2,67 g, m. p. 265—266°C (decomp.). The obtained substance was dissolved in 9 ml of hot water, filtered and crystallized by adding of 40 ml of ethanol, allowed to stand for 3 hours at 0°C, filtered and washed with waterfree ethanol and ether, yield 2,26 g of pure D-hydroxy-prolin (65%), m. p. 274—275°C (decomp.). $[\alpha]_D^{20} = +77°$ (c: 1,001; H_2O) [2], [3].

3,5-Dinitrobenzoyl-L-hydroxy-prolin. Mother liquor obtained from resolvation combined with the washing alcohol of D-brucinate was inoculated with 3,5-dinitrobenzoyl-L-hydroxy-prolin brucinate and the solution was kept for 8 hours at 0°C. The separated crystals were filtered without washing and dried, yield 15,27 g. Recrystallization of the filtered product from 120 ml of water after cooling for 8 hours gave 12,4 g (80%) pure, yellow colored 3,5-dinitrobenzoyl-L-hydroxy-prolin, m. p. 95—108°C. $[\alpha]_D^{20} = -50°$ (c: 1,020; abs. ethanol).

The obtained amount (12,4 g) of L-brucinate was treated further as described under the heading of the D-modification, to afford 5,14 g (93%) of solid foam 3,5-dinitrobenzoyl-L-hydroxy-prolin. $[\alpha]_D^{20} = -145°$ (c: 1,112; 50% ethanol).

L-Hydroxy-prolin (II). Hydrolysis with 5 N hydrochloric acid of the product obtained in the previous step was fulfilled as described under the heading of the D-modification, to afford 1,72 g crude L-hydroxy-prolin, m. p. 264—265°C (decomp.). The substance was dissolved in 5 ml of hot water, filtered and crystallised by adding of 30 ml of ethanol, allowed to stand for 8 hours at 0°C, filtered and washed with waterfree ethanol and ether, yield 1,34 g of pure L-hydroxy-prolin (61%), m. p. 273—274°C (decomp.). $[\alpha]_D^{20} = -76°$ (c: 1,001; H_2O) [1], [4].

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